

Figure 1: Human POSH Coding Sequence (SEQ ID NO:1) (part 1)

ATGGATGAATCAGCCTTGTGGATCTTTTGGAGTGTCCGGTGTGTCTAGAGCGCCTTGATGCTTCTGCGA
AGGTCTTGCCCTTGCCAGCATACGTTTTTGCAAGCGATGTTTGTCTGGGGATCGTAGGTTCTCGAAATGAACT
CAGATGTCCCGAGTGCAGGACTCTTGTGGCTCGGGTGTGAGGAGCTTCCAGTAACATCTTGCTGGTC
AGACTTCTGGATGGCATCAAACAGAGGCCTTGGAAACCTGGTCTGGTGGGGGAAGTGGGACCAACTGCA
CAAATGCATTAAGGTCTCAGAGCAGCACTGTGGCTAATTGTAGCTCAAAGATCTGCAGAGCTCCCAGGG
CGGACAGCAGCCTCGGGTGAATCCTGGAGCCCCCAGTGAGGGGTATACCTCAGTTACCATGTGCCAAA
GCGTTATACAACATATGAAGGAAAAGAGCCTGGAGACCTTAAATTCAGCAAAGGCGACATCATCATTTTGC
GAAGACAAGTGGATGAAAATTGGTACCATGGGGAAGTCAATGGAATCCATGGCTTTTTTCCCACTT
TGTGCAGATTATTAAACCGTTACCTCAGCCCCACCTCAGTGCAAAGCACTTTATGACTTTGAAGTGAAA
GACAAGGAAGCAGACAAAGATTGCCTTCCATTTGCAAAGGATGATGTTCTGACTGTGATCCGAAGAGTGG
ATGAAAACCTGGGCTGAAGGAATGCTGGCAGACAAAATAGGAATATTTCCAATTTTCATATGTTGAGTTTAA
CTCGGCTGCTAAGCAGCTGATAGAATGGGATAAGCCTCCTGTGCCAGGAGTTGATGCTGGAGAATGTTCC
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CCCCCTGTCTCATCAGCTCCAGCAACCCCACTGCTGCTGCACGGATCAGCGAGCTGTCTGGGCTCTCC
TGCACTGCCCCCTTCTCAGGTTTCATATAAGTACCACCGGGTTAATGTGACCCCGCCCCCAAGCAGCCAG
TGACAACCTGGCCCCCTCGTTTACTTTCCCATCAGATGTTCCCTACCAAGCTGCCCTTGGAACTTTGAATCC
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ATGGCTGGTTCAAAGGCACATTACAACGTAATGGGAAAACCTGGCCTTTTCCAGGAAGCTTTGTGGAAAA
CATATGA

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Figure 2: Human POSH Amino Acid Sequence (SEQ ID NO:2) (part 2)

MDESALLDLLECPVCLERLDASAKVLPCQHTFCKRCLLGIVGSRNELRCPECRTLVGSGVEELPSNILLV
RLLDGIKQRPWKPGPGGGSGTNCNALSQSSTVANCSSKDLQSSQGGQOPRVQSWSPVVRGIPQLPCKAK
ALYNYEGKEPGDLKFSKGDIIILRRQVDENWYHGEVNGIHGFFPTNFVQIIKPLPQPPPQCKALYDFEVK
DKEADKDCLPFAKDDVLTVIRRVNENWAEGLADKIGIFPISYVEFNAAKQLIEWDKPPVPGVDAGECS
SAAQSSSTAPKHSDTKKNTKKRHSFTSLTMANKSSQASQNRHSMEISPPVLISSNPTAAARISELSGLS
CSAPSQVHISTTGLIVTPPPSSPVTGTPSFTFPPSDVPYQAALGTLNPPPLPPPLLAATVLAATPPGATAA
AAAAGMGPRPMAGSTDQIAHLRPQTRPSVYVAIYPYTPRKEDELELRKGEMFLVFERCQDGFKGTSMHT
SKIGVFPNGYVAPVTRAVTNASQAKVPMSTAGQTSRGVTMVSPSTAGGPAQKLQNGVAGSPSVVPAVV
SAAHIQTSPQAKVLLHMTGQMTVNQARNVTRVAAHNQRPTAAVTPIQVQNAAGLSPASVGLSHHSLAS
PQPAPLMPGSATHTAAISISRASAPLACAAAAPLTSPSITSASLEAEPSGRIVTVLPGLPTSPDSASSAC
GNSSATKPKDKSKKEKKGLLKLKLSGASTKKRKPVSPPASPTLEVELGSAELPLQGAVGPPELPPGGGHGRA
GSCPVDGDPVTTAVAGAALAQDAFHRKASSLDSAVPIAPPPRQACSSLGTVLNESEPPVVCERHRVVVSY
PPQSEAELELKEGDIVFVHKKREDGWFKGTLQQRNGKTGLFPGSFVENI

Figure 3: Human POSH cDNA Sequence (SEQ ID NO:3)

CTGAGAGACACTGCGAGCGGCGAGCGGGTGGGGCCGCATCTGCATCAGCCGCCGAGCCGCTGCGGGGC
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 CTTGTTGGATCTTTTGGAGTGTCCGGTGTGTCTAGAGCGCCTTGATGCTTCTGCGAAGGTCTTGCCCTGTC
 CAGCATACGTTTTTGCAGCGATGTTTGTGCGGGATCGTAGGTTCTCGAAATGAACTCAGATGTCCCGAGT
 GCAGGACTCTTGTGGCTCGGGTGTGCGAGGAGCTTCCAGTAACATCTTGCTGGTCAGACTTCTGGATGG
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 TCTCAGAGCAGCACTGTGGCTAATTGTAGCTCAAAGATCTGCAGAGCTCCAGGGCGGACAGCAGCCTC
 GGGTGAATCCTGGAGCCCCCAGTGAGGGGTATACCTCAGTTACCATGTGCCAAAGCGTTATACAACATA
 TGAAGGAAAAGAGCCTGGAGACCTTAAATTCAGCAAAGGCGACATCATCATTTTTCGGAAGACAAGTGGAT
 GAAAATTGGTACCATGGGGAAGTCAATGGAATCCATGGCTTTTTCACCAACTTTGTGCAGATTATTA
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 CAAAGATTGCCCTTCCATTTGCAAAGGATGATGTTCTGACTGTGATCCGAAGAGTGGATGAAAACCTGGCT
 GAAGGAATGCTGGCAGACAAAATAGGAATATTTCCAATTTTCATATGTTGAGTTTAACTCGGCTGCTAAGC
 AGCTGATAGAAATGGGATAAGCCTCCTGTGCCAGGAGTTGATGCTGGAGAATGTTCTCGGCAGCAGCCCA
 GAGCAGCACTGCCCAAAGCACTCCGACACCAAGAAGAACACCAAAAAGCGGCACTCCTTCACTTCCCTC
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 TCAGGTTTCATATAAGTACCACCGGTTAATTGTGACCCCGCCCCAAGCAGCCAGTGAACAATGGCCCC
 TCGTTTACTTTTCCCATCAGATGTTCCCTACCAAGCTGCCCTTGGAACTTTGAATCCTCTCTTCCACAC
 CCCCTCTCTGCTGCCACTGTCTTGCCTCCACACCACAGGCGCACCGCCGCGCTGTCTGTCTGTGG
 AATGGGACCGAGGCCCATGGCAGGATCCACTGACCAGATTGCACATTTACGGCCGAGACTCGCCCCAGT
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 TTTCTCTGGCAATATGTGGCACCACTCACAAGGGCGGTGACAAATGCTTCCCAAGCTAAAGTCCCTATG
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 ATGCCCGCCGCTCAGCCCTGCATCTGTGGCCTGTCCCATCACTCGCTGGCCTCCCAACAACCTGGCC
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 GCAGCAGCTGCTCCACTGACTTCCCAAGCATCACCAGTGTCTCTGGAGGCTGAGCCAGTGGCCGGA
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 TGGGTTTTTAAATTTCTAGAAATGAAGTGAATGAACAATGAGAAAGAAATACAGCACACCCTTGAACAA
 AATGTATTTAGAAATATATTTAGTTTATAGCAGAAGCAGTCAATTTGTTTGGTTGGAAGTAGGGGAAA
 TTGAAGTTGTAGTCACTGTCTGAGAATGGCTATGAAGCGTCATTTACATTTTACCCCAACTGACCTGCA
 TGCCAGGACACAAGTAAACATTTGTGAGATAGTGGTGGTAAGTGATGCACTCGTGTAAAGTCAAAGGC
 TATAAGAAACACTGTGAAAAGTTCATATTCATCCATTGTGATTCTTTCCACGCTTTCATGTATTACT
 GGATTCCACAGTAAATATAGACTGTGCATGGTGTATATTTTATGCGATTTCCTGTTAAGATGAGTTT
 GTACTCAGAATTGACCAATTACAGAGGTGTAATAATAACAGTGTCTCTCTTCTACCCCAAAGCCACTA

-to be continued

Figure 3: Human POSH cDNA Sequence (SEQ ID NO:3)

CTGACCAAGGTCTCTTCAGTGCACTCGCTCCCTCTCTGGCTAAGGCATGCATTAGCCACTACACAAGTCA
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CTGTGACTGTGGAGCTCTGGAAGGCTTGGTGGGAGTGAATTTGCCACACCTTACAATTGTGGCAGGATC
CAGAAGAGCCTGTCTTTTATATCCATTCTTGATGTCAATGGCCTCTCCACCGATTTTCATTACGGTGC
CACGCAGTCATGGATCTGGGTAGTCCGGAAAACAAAAGGAGGGAAGACAGCCTGGTAATGAATAAGATCC
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CTGGTATAAAGTTGGTTAAAATTTTCATCTGTTAATAGATCATTAGGTAATATAATGTATGGGTTTTCTAT
TGGTTTTTTTGCAGACAGTAGAGGGAGATTTTGTAAACAAGGGCTTGTACACAGTGATATGGTAATGATAA
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TGATGTTCAAACCTTTGT

Figure 4: 5' cDNA fragment of human POSH (public gi:10432611; SEQ ID NO:4)

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MDESALLDLLECPVCLERLDASAKVLPQCHTFCKRCLLGIVGSRNELRCPECRTLVGSGVEELPSNILLV
RLLDGIKQRPWKPGPGGGSGTNTNALRSQSSTVANCSSKDLOSSQGGQQPRVQSWSPFVRGIPQLPCA
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DKEADKDCPLPFAKDDVLTVIRRVDENWAEGLMADLKIGIFPISYVEFNSSAAKQLEIWDKPPVPGVDAGECS
SAAQSQSTAPHKSDTKTKNTKKRHSPTGLTMAKSSSQASQNRHSMELSPVLISSNPTAAARISSELSGLS
CSAPQSVHISTTGLTVTPPPSSPTTGSFTFSPDVPYQALGTLNPLPLPPPLLAATVLASTPPGATAA
AAAAGMGRPRMAGSTDQIAHLRPQTRPSVYVAIYPYTPRKEDELELRKGEMFLVFERCQDGFVKGTSMHT
SKIGVFPNGYVAPVTRAVTNASQAKVPMSTAGQTSRGVTMVSPSTAGGPAQKLQNGVAGSPSVVPAAV
SAAHIQTSPOAKVLLHMTGQMTVNQARNAVRTVAAHNQERPTAAVTPIQVQNAAGLSPASVGLSHHSLAS
PQPAPLMPGSATHTTAISISRASAPLACAAAAPLTSPSISASLEAEPSSGRIVTVLPLGLTPSPDSASSAC
GNSSATKPKDKDS

Figure 6: 3' mRNA fragment of hPOSH (public gi:7959248; SEQ ID NO:6)

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 gctatgtgattgaagtacctctgttcatagtttctcgtgtataaagttgggttaaaatttcatctgttaata
 gatcattaggtataataatgtatgggttttctattgggtttttgcagacagtagaggagattttgtaac
 aagggttgttacacagtgatattggtaataaatttgcaatttatcactcctttcatgttaataatt
 tgaggactggataaaaagggttcaagataaaaattgcatgttcaaacctttgt

Figure 7: C terminus protein fragment of hPOSH (public gi:7959249; SEQ ID NO:7)

ISYVEFN SAAKQLIEWDKPPVPGVDAGECSSAAQSS TAPKHS DTKKNTKKRHSFTSLTMANKSSQASQN
RHSMEISPPVLISSSNPTAAARISELSGLSCSAPSQVHISTTG LIVTPPPSSPVTTGPSFTFPDVPYQA
ALGTLNPPPLPPPPLLAATV LASTPPGATAAAAAAGMGRP MAGSTDQIAHLRPQTRPSVYVAIYPYTPRK
EDELELRKGEMFLVFERCQDGWFKGTS MHTSKIGVFP GNYVAPVTRAVTNASQAKVPMSTAGQTSRGVTM
VSPSTAGGPAQKLQGNVAGSPSVVPAAVVSAAHIQTS PQA KVL LHM TGQMTV NQARN AVRTVA AHNQER
PTAAVTPIQVQNAAGLSPASVGLSHHSLAS PQAPLMPGSATHTA AISISRASAPLACAAAAPLTSPSIT
SASLEAEPSGRIVTVLPGLPTSPDSASSACGNSSATKPKDKSKKEKKGLLKL LSGASTKRKPRVSPASP
TLEVELGSAELPLQGA VGPELP PGGGHGRAGSCPVDGDGPVTTAVAGAA LAQDAFHRKASSLDSAVPIAP
PPRQACSSSLGPVLNESRPVVCERHRVVVSYP PQSEAELELKEGDIVFVHKKREDGWFKGTLQRNGKTGLF
PGSFVENI

Figure 8: Human POSH full mRNA, Annotated Sequence (part 1)

```

---- - gi|10432611|dbj|AK021429.1|AK021429 Homo sapiens cDNA
FLJ11367 fis, clone HEMBA1000303, highly similar to Mus musculus
Plenty of SH3s (POSH) mRNA

```

----- gi|7959248|dbj|AB040927.1|AB040927 Homo sapiens mRNA for KIAA1494 protein, partial cds

-- -- - Both hPOSH and KIAA1495

~~Ring~~ - Ring Domain

SH3 Domian

 - start codon and stop codon of predicted ORF

[illegible]

-to be continued

Figure 8: Human POSH full mRNA, Annotated Sequence (part 2)

TTGTGGACTTCCAGATGGTCAGGAGATGAGCAAAGGATTGGTATGTGACTCTGATGCCCCAGCACAGTTA
 CCCCAGCGAGCAGAGTGAAAGATGTTTGTGTGGGTTTGTAGTCTGGATTCGGATGTATAAGGTGTG
 CCTTGTAAGTGTCTGATTTACTACACAGAGAACTTTTTTTTTTTTAAAGATATAGACTAAATGGACA
 ATTGTTTACAAGGCTTAATAATTTATTTGCTTTTTTAAACTTGAACTTTTCGTATAATAGATACGTTCT
 TTGGATTATGATTTTAAGAAATTATTAATTTATGAAATGATAGGTAAGGAGAAGCTGGATTATCTCCTGT
 TGAGAGCAAGAGATTCGTTTGGACATAGAGTGAATGCATTTCCCTCTCCTCCTCCTGCTACCATTAT
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 CAGAAGAGCCTGTCTTTTATATCCATTCTTGTATGTCATTGGCCTCTCCCACTGATTTCATTACGGTGC
 CACGCAGTCATGGATCTGGGTAGTCCGGAACAAAGAGGGAAGACAGCCTGGTAATGAATAAGATCC
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 AATTGCAATTTATCACTCCTTTTCATGTTAATAATTTGAGGACTGGATAAAAGGTTTCAAGATTAAATTT
 TGATGTTCAAACCTTTGT

Figure 9: Domain Analysis of Human POSH

Domain Name	begin	end	E-value
<u>RING</u>	12	52	1.06e-08
<u>SH3</u>	137	192	2.76e-19
<u>SH3</u>	199	258	4.84e-15
<u>low complexity</u>	366	384	-
<u>low complexity</u>	390	434	-
<u>SH3</u>	448	505	2.40e-19
<u>low complexity</u>	547	563	-
<u>low complexity</u>	652	668	-
<u>low complexity</u>	705	729	-
<u>SH3</u>	832	888	1.47e-14

Figure 10: Diagram of Human POSH Nucleic Acids

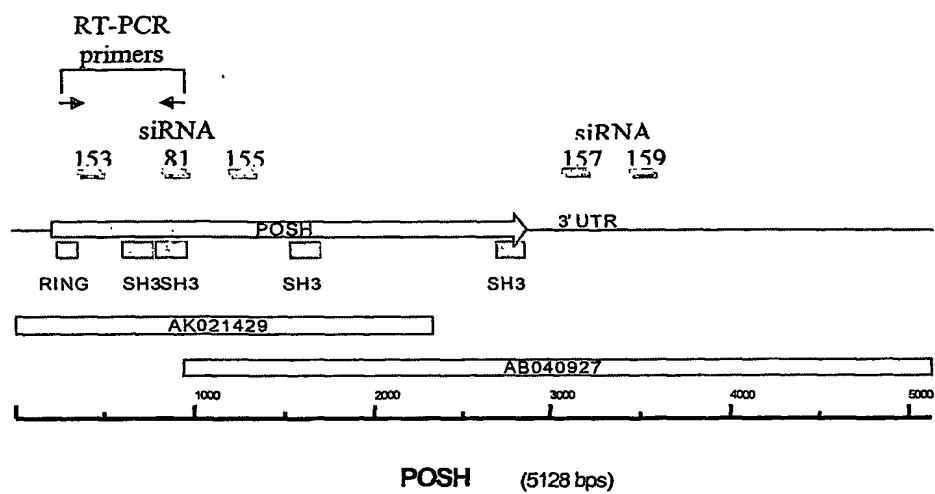


Figure 11: Reduction in Full Length POSH mRNA by siRNA Duplexes

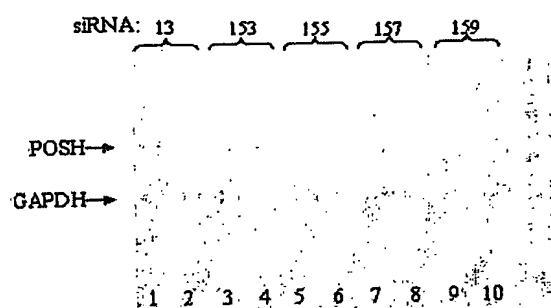


Figure 12: POSH Affects Release of VLP from Cells

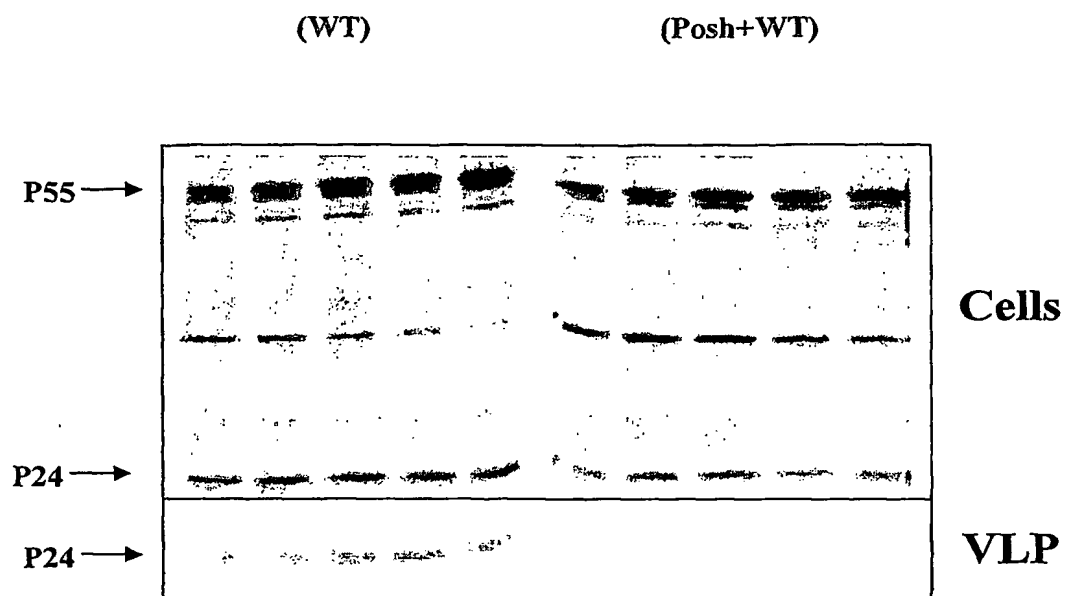
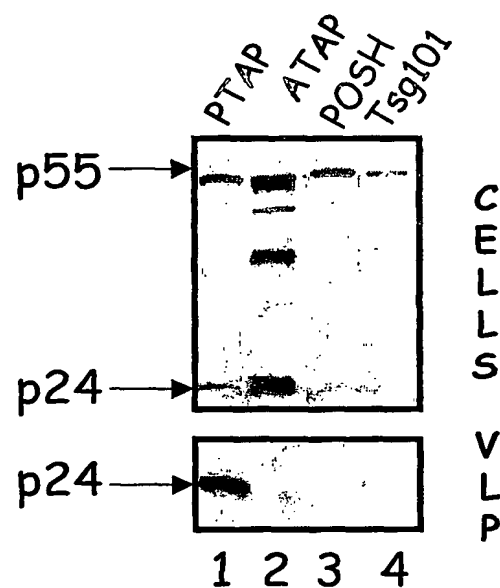


Figure 13: Release of VLP from Cells at Steady State



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GGCTCGAAAGATCTGCAGAGCTCCAGTGTGGACAGCAGCTCGGGTGCAAGCCTGGAGCCCCCAGTGA
GGGAATACCTCAGTTACCGTGTGCGCAAAAGCATATATAACTACGAAGGAAAAGACGCCGGAGACCTTAA
GTTTCAGCAAAGGCGACACCATCTTGCGCCGACAGGTGGATGAGAATTTGGTACACCGGGGAAGTCAG
GGGGTCCACGGCTTTTTCCCACTAACTTCTGTGCAGATCATCAAACCTTTACCTCAGCCCCCGCTCAGT
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TGCCAGGAGTGGACACGGCAGATGCCCTCAGCGACGGCGCAGAGCACCTCTGCCTCAAAGCACCCCGA
ACCAAGAAGAACCACAGGAAGCGACACTCCTTCACTCCCTCACCATTGSCCAACAAGCTTCTCCAGGGG
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GTTGCTATATATCCGTACACTCCCCGGAAGGAAGACGAACCTGGAGCTGAGGAAAGGGGAGATGTTTTTGG
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CAAGGAAGGAGATATTTGTTTTGTTTCTATAAGAAACGAGAGGCGGCTGGTTCAAAGGCACGTTACAGAG
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Figure 15: Mouse POSH Protein sequence (Public gi: 10946922; SEQ ID NO: 9)

MDESALLDLLECPVCLERLDASAKVLPCHQTFCKRCLLGIVGSRNELRCPECRTLVGSGVDELPSNILLV
RLLDGIKQRPWKPGPGGGGGTTCTNTLRAQGSTVVNCGSKDLQSSQCGQQPRVQAWSPVVRGIQQLPCAK
ALYNYEGKEPGDLKFSKGDITILRRQVDENWYHGEVSGVHGFFPTNFVQIIKPLPQPPPOCKALYDFEVK
DKEADKDCLPFAKDDVLTIVIRRVDENWAEGLADKIGIFPISYVEFNAAKQLIEWDKPPVPGVDTAECF
SATAQSTSASKHPDTKKNTRKRHSFTSLTMANKSSQGSQNRHSMEISPPVLISSSNPTAAARISELSGLS
CSAPSQVHIISTGLIVTPPPSSPVTTGPAFTFSPDVYQAALGSMNPPLPPPPLLAATVLAATPSGATAA
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KGTSMHTSKIGVFPNGYVAPVTRAVTNASQAKVSMSTAGQASRGVTMVSPSTAGGPTQKPQNGVAGNPS
VVPTAVVSAAHIQTSPOAKVLLHMSGQMTVNQARNAVRTVAAHSQERPTAAVTPIQVQNAACLGPAVGL
PHHSLASQPLPPMAGPAAHGAAVSISRTNAPMACAAGASLASPNMTSAMLETPSGRTVTIILPGLPTSPE
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GSHGRVGSCTDGDGPVAAAGTAALAQDAFHRKTSSLDSAVPIAPPPRQACSSLGPVMNEARPVVCERHRV
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Figure 16: *Drosophila melanogaster* POSH mRNA sequence (public gi:17737480; SEQ ID NO:10)

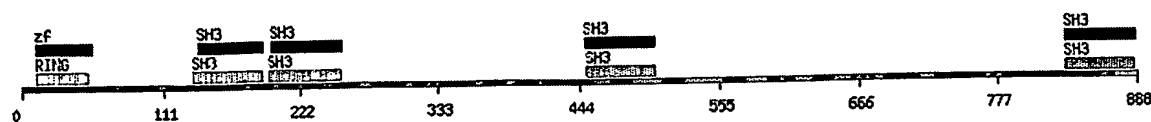
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Figure 17: *Drosophila melanogaster* POSH protein sequence (public gi:17737481; SEQ ID NO:11)

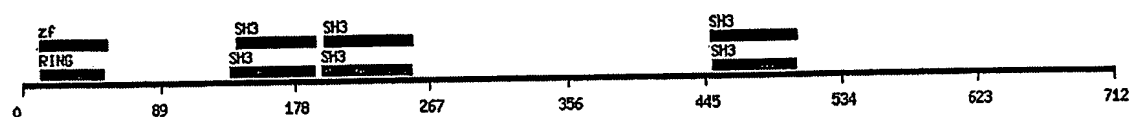
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KEKRHSLNALLGGGAPLSLLQTNRHSAEILSLPHELRLVSSSTALKPTSAPQTSRVLKTTVQQQMOPN
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Figure 18: POSH Domain Analysis

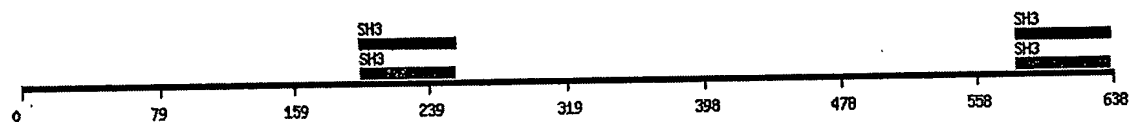
hPOSH protein sequence :



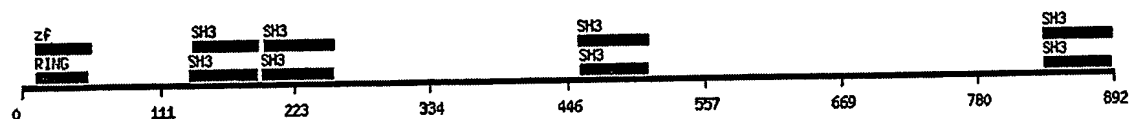
N terminus protein fragment of hPOSH (public gi:10432612):



C terminus protein fragment of hPOSH (public gi:7959249):



Mouse POSH Protein sequence (Public gi: 10946922):



Drosophila melanogaster POSH protein sequence (public gi:17737481)



Figure 19: Human POSH has ubiquitin ligase activity

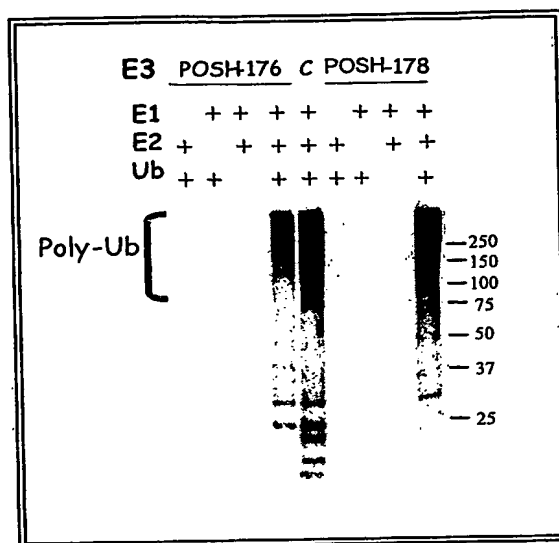


Figure 20.

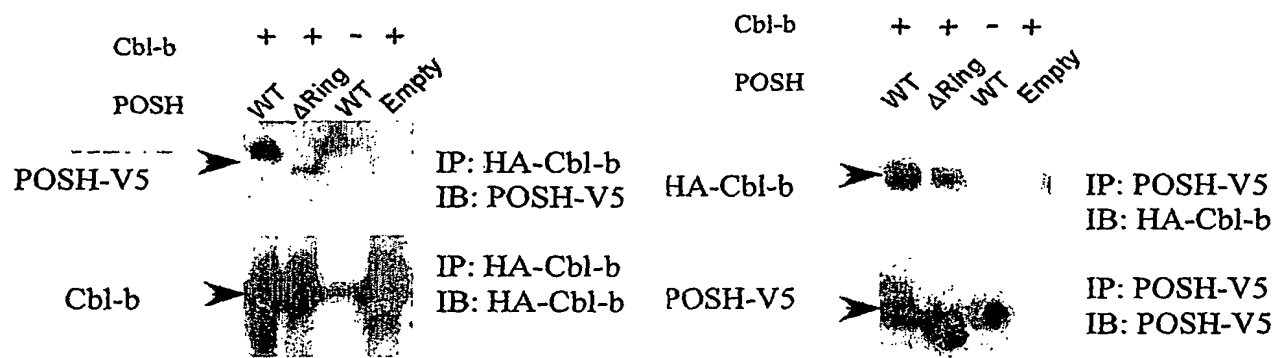


Figure 21. PLD activity in medium of transfected cells

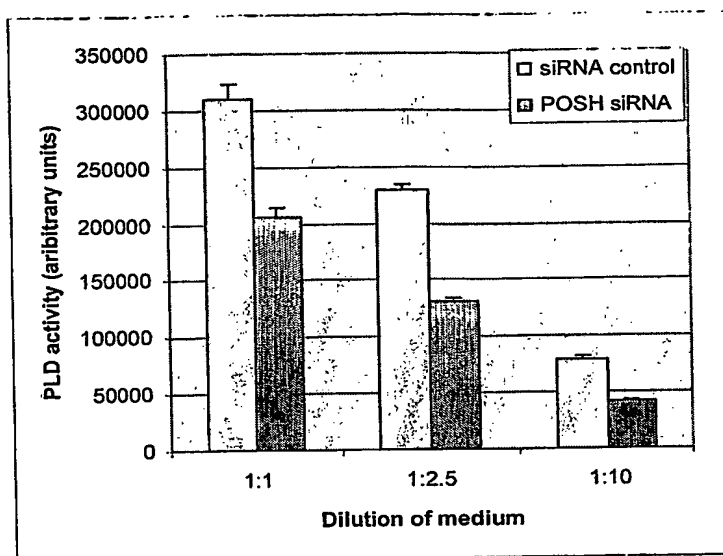


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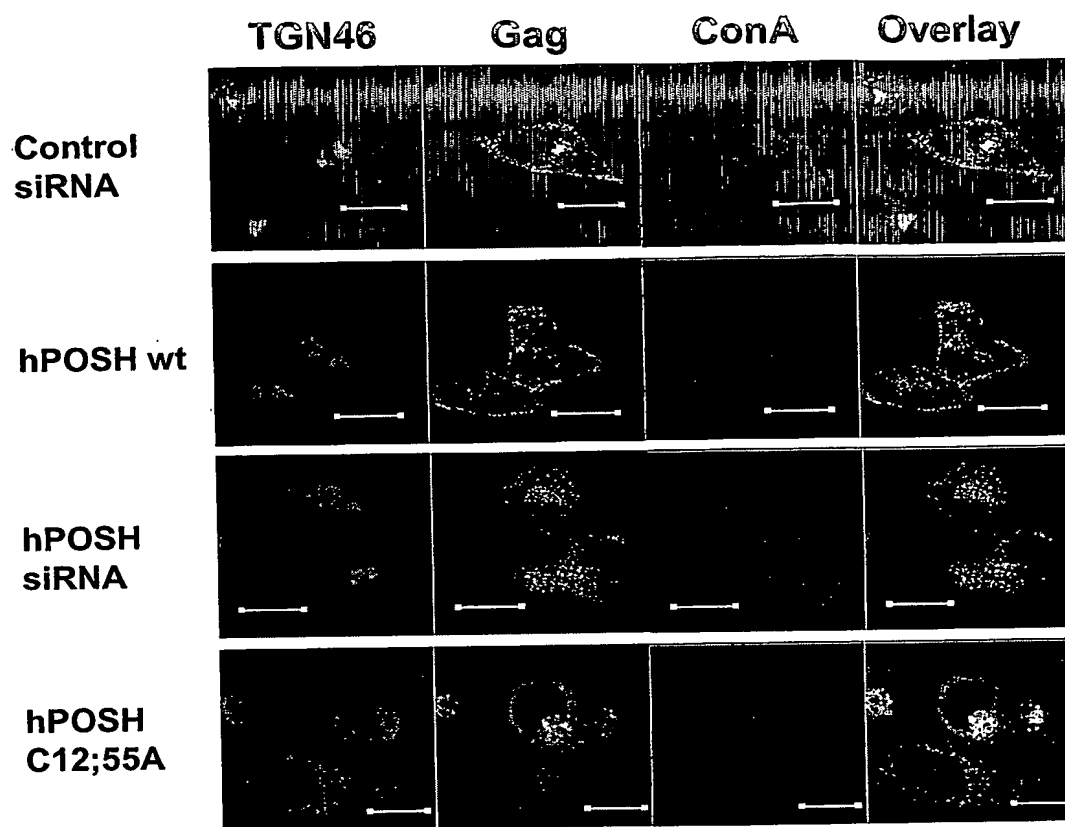


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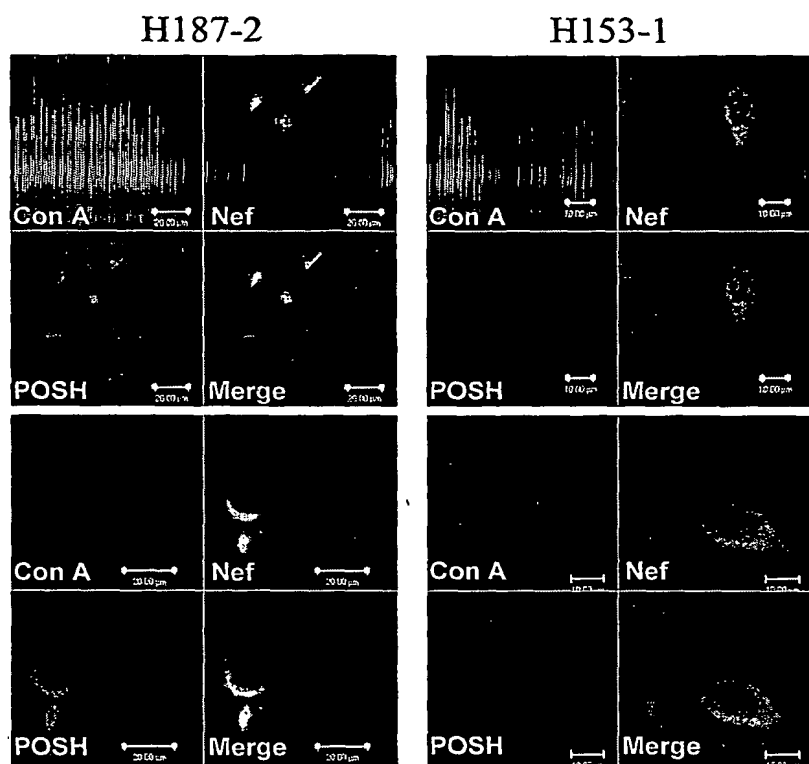


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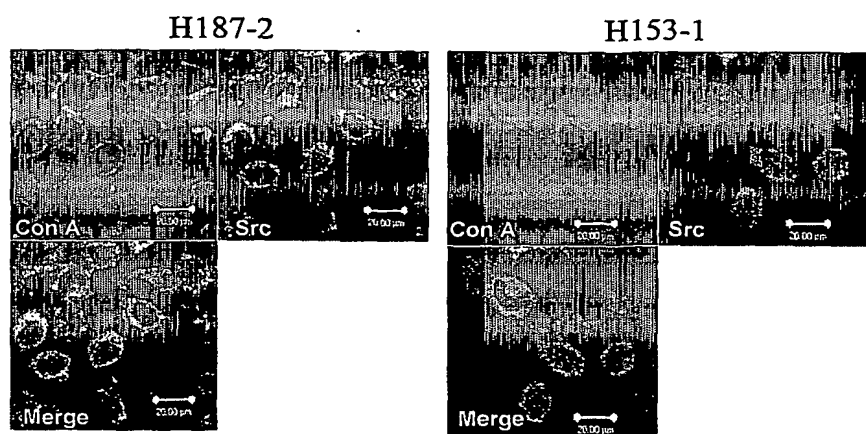
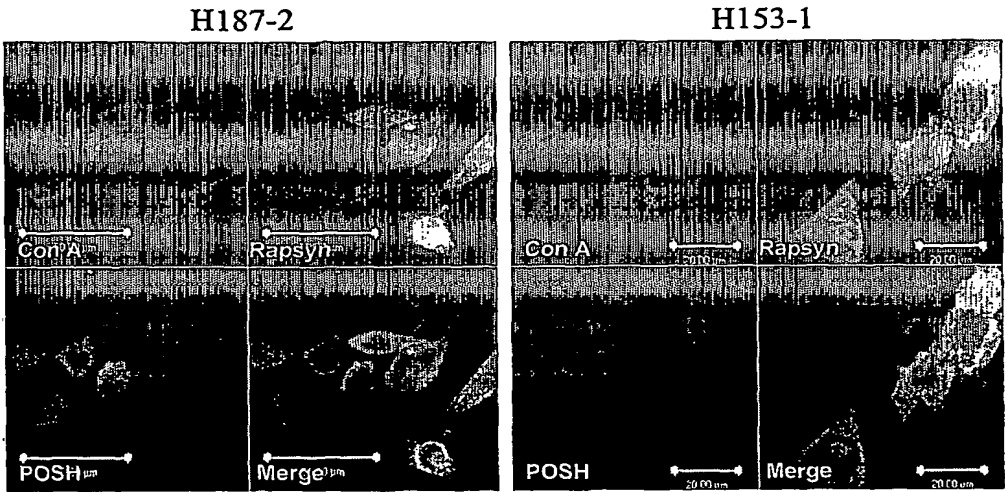


Figure 25.



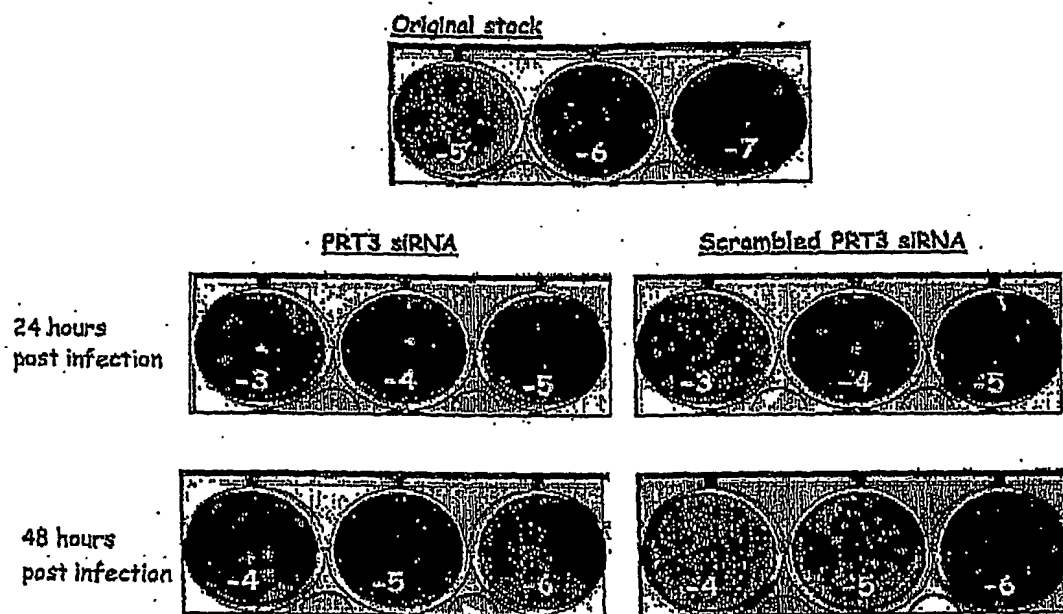


FIGURE 26

Figure 27.

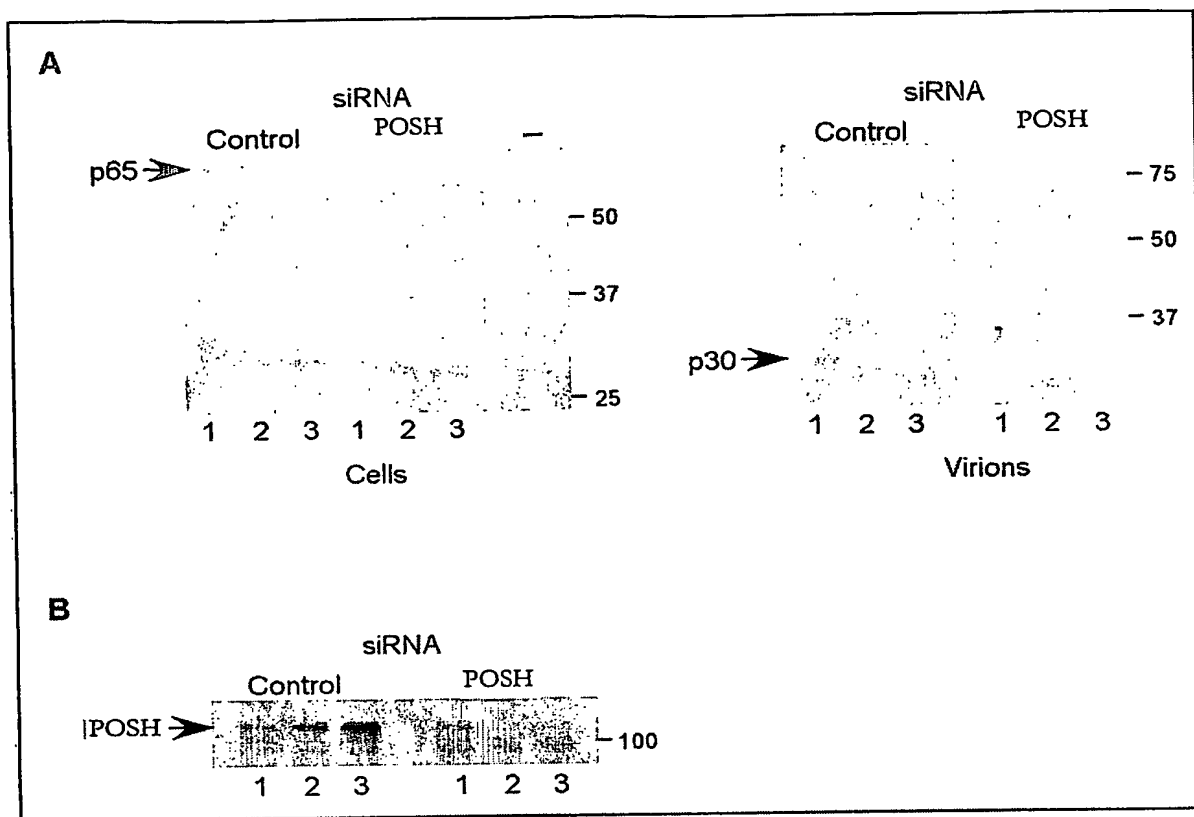


Figure 28.

SiRNA-Tsg101

SiRNA-POSH

Control

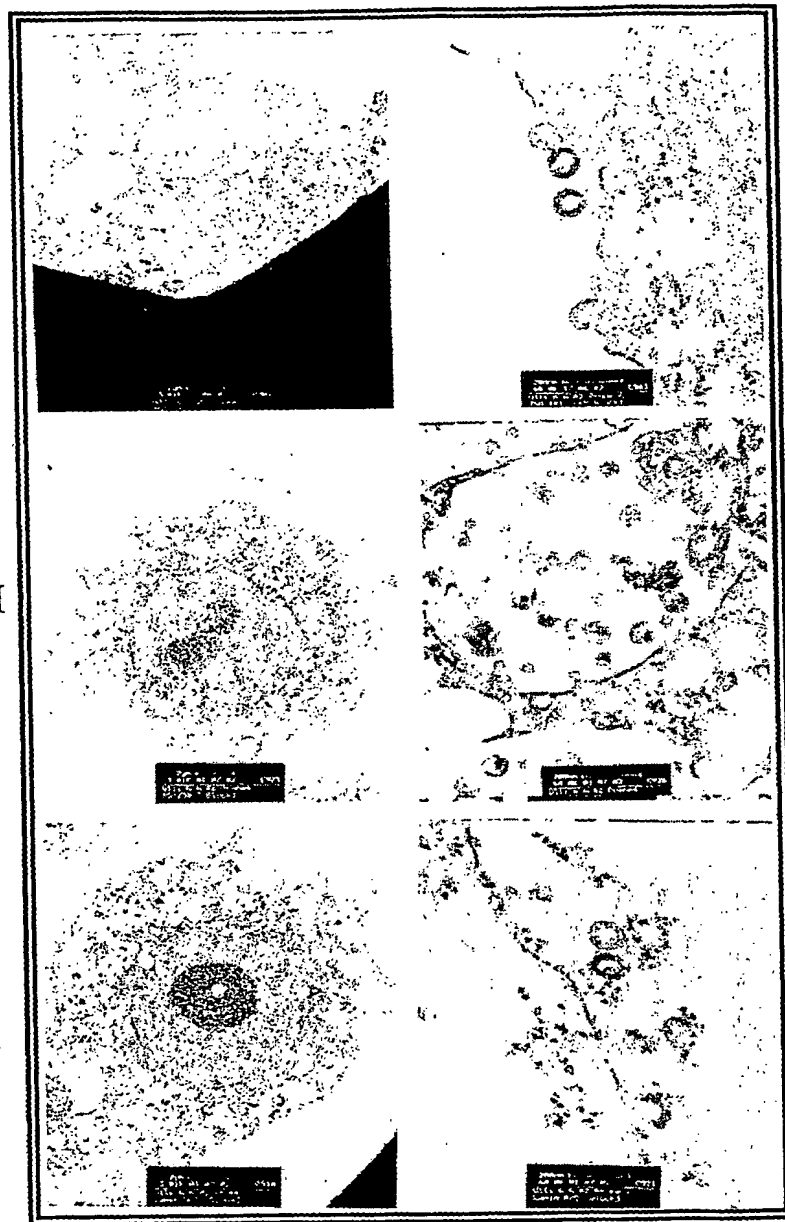


Figure 29.

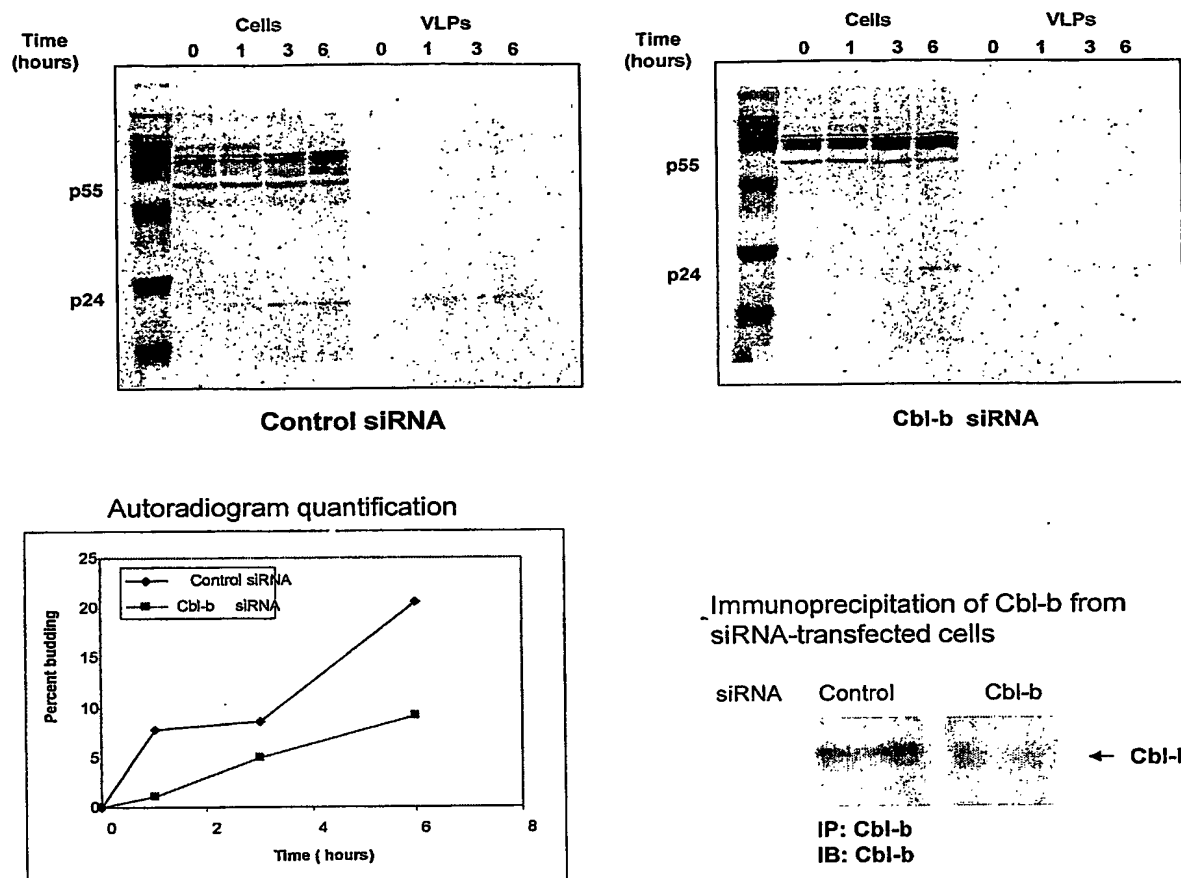


Figure 30.

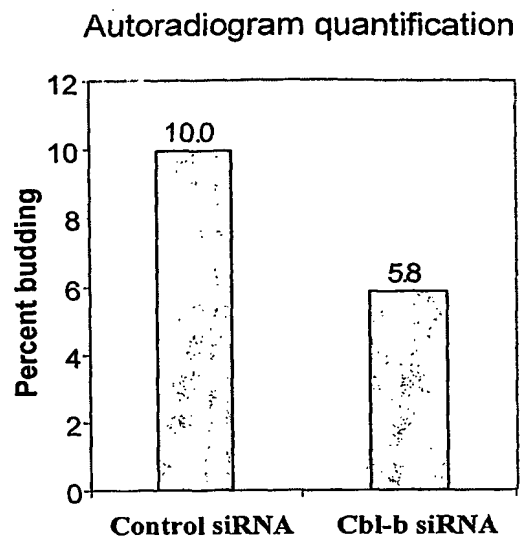
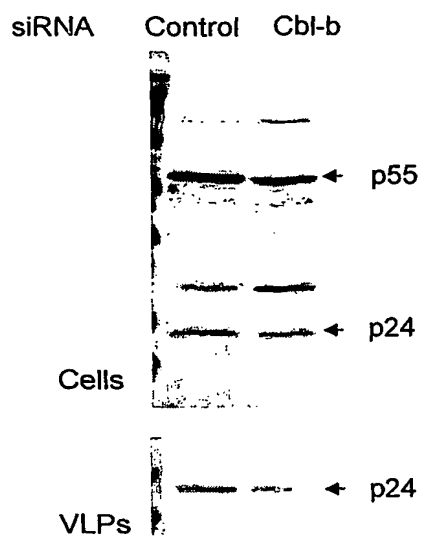


Figure 31. RT activity in VLP secreted from cells treated with indicated siRNAs.

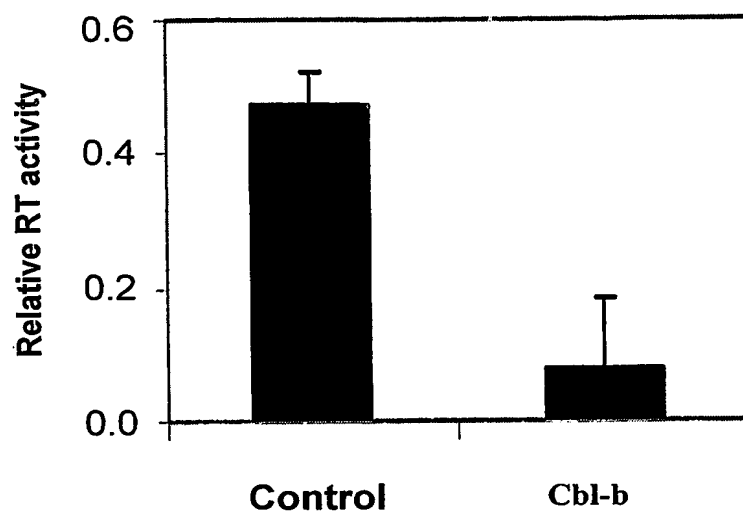
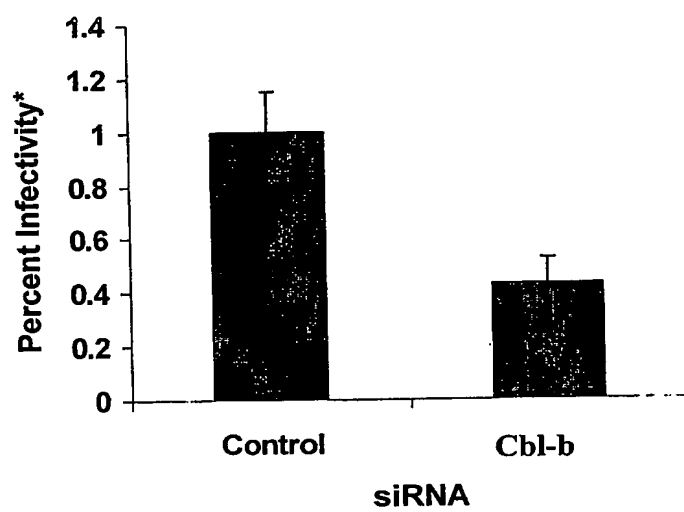


Figure 32. HIV-1 infectivity assay in cells treated with siRNA against Cbl-b.



* (normalized to control)

Figure 33. RT activity in VLP secreted from cells transfected with indicated plasmids.

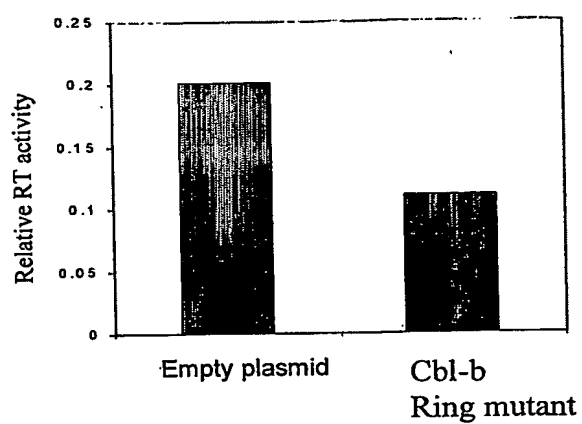
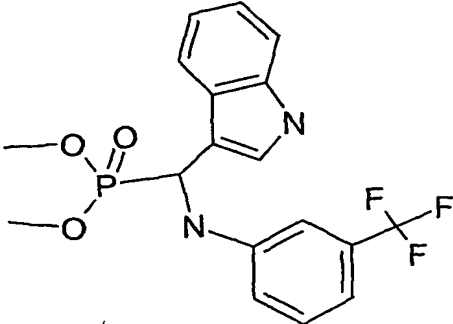
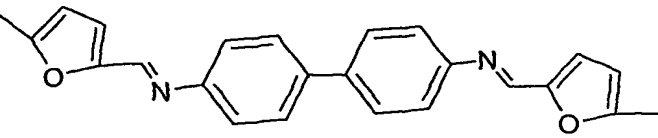
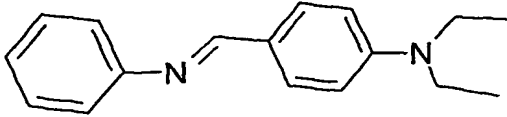
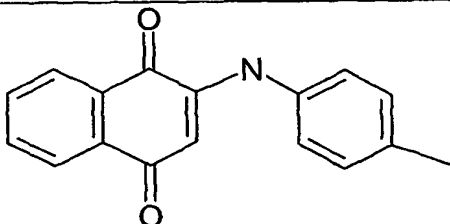
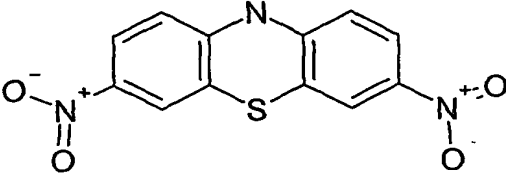
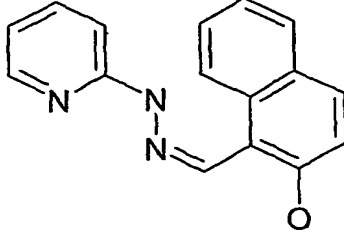
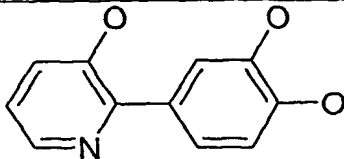
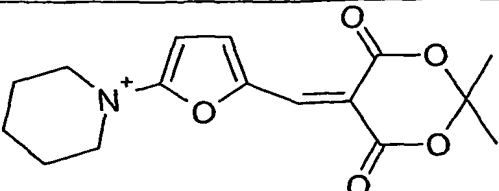
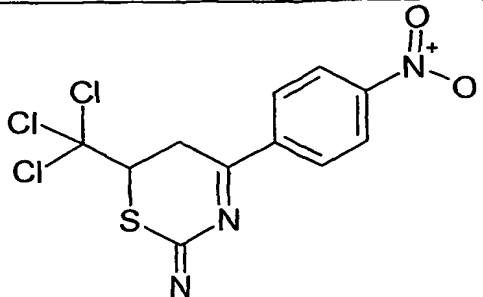
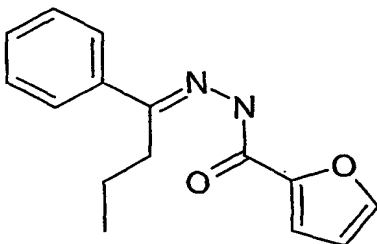
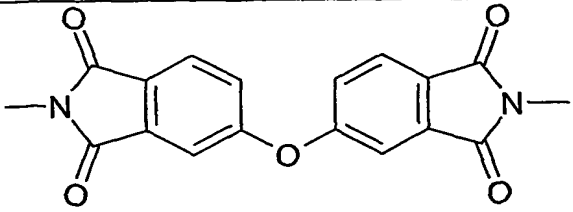
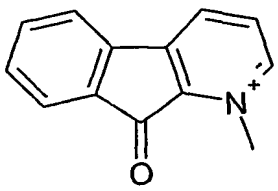
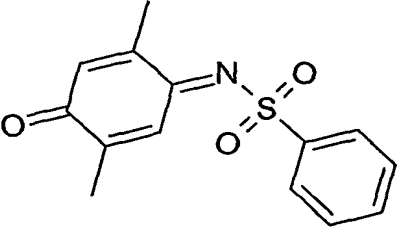


Figure 34.

CAS	MW (gr/mol)	Structure	%A
412945-52-9	398. 33		48.7928
52686-41-6	368. 44		36.7342
38536-86-6	252. 36		25.4204
57182-49-7	263. 3		33.8353
63245-76-1	289. 27		5.22885
120999-01-1	263. 3		42.2514
126324-76-3	203. 2		25.1252

164399-38-0	386. 25		35.0247
324526-59-2	352. 63		20.7212
295345-11-8	256. 31		37.633
no cas	336. 31		37.2901
325958-44-9	323. 14		27.7748
88680-99-3	275. 33		23.2871

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